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dCAF-1-p55 is essential for *Drosophila* development and involved in the maintenance of chromosomal stability*

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Abstract The chromatin assembly factor 1 (CAF-1) is a three-subunit protein complex that functions in chaperoning histone deposition. The smallest subunit of *Drosophila* CAF-1, dCAF-1-p55, together with the other two subunits dCAF-1-p180 and dCAF-1-p105, participates in the deposition of histone H3 and H4 onto the newly synthesized DNA. In addition to the CAF-1 complex, dCAF-1-p55 is also found in several other complexes such as NURF, PRC2 and Sin3-HDAC1 complex, suggesting that it plays multiple roles other than a histone chaperone. Here we report a new allele of *dCAF-1-p55* generated by gene targeting. We show that *dCAF-1-p55* loss-of-function led to developmental delay and lethality. Further investigations demonstrated that *dCAF-1-p55* deficient cells exhibited defects in metaphase chromosome condensation, sister chromatids association and anaphase chromosomes segregation, which are premonitory signs of chromosome instability (CIN). Taken together, these results indicate that *dCAF-1-p55* plays a role in the maintenance of chromosomal stability, likely to protect the cells from CIN and the proneness to cancer.
Key words *Drosophila, dCAF-1-p55,* chromosome instability, chromosome segregation, sister chromatids association.

Chromosomal instability (CIN) is a type of genomic instability which refers to a consistent high rate of chromosomal gains and losses [1]. Abnormalities during chromosome segregation, inculding defects in chromosome cohesion, spindle assembly checkpoint (SAC), centrosome copy number, kinetochore–microtubule attachment dynamics and cell-cycle regulation, can lead to CIN [2]. As a hallmarker of cancrer, CIN often results in increased frenquency of lagging chromosomes in the anaphase during cell division [3-4].

CAF-1, the well-known chromatin assembly factor 1, is involved in the deposition of H3/H4 onto the newly synthesized DNA. In *Drosophila*, dCAF-1 is a three-subunit complex containing dCAF-1-p180, dCAF-1-p105, and dCAF-1-p55 [5-7]. As the smallest subunit of dCAF-1, dCAF-1-p55 is conserved from plants to humans (MSI1-5 in plants, LIN53 in worms, and RbAp48/46 in humans). Among them, *Drosophila* dCAF-1-p55 shows over 84% identity with human RbAp48/46 [8]. In *Arabidopsis*, *MSI1* loss-of-function leads to flowering time defect [9], while *MSI4* and *MSI5*, have been demonstrated to play a role in the epigenetic regulation of chromatin silencing [10]. In *C. elegans,* mutants of *lin53* show multivulva phenotype [11]. Knock-down of *dCAF-1-p55* in *Drosophila* S2 cells alters the expression levels of dE2F2 target genes [12]. *In vitro* experiments showed that dCAF-1-p55 participates in CENP-A (CID in *Drosophila*) assembly [13-15]. The dCAF-1-p55 homolog in humans, RbAp48/46,

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was initially identified as a retinoblastoma (Rb) associated protein [16-17]. RbAp48/46 has been found to be expressed abnormally in various cancers, including breast cancer, human primary hepatocellular carcinoma and cervical cancer [18-21].

In addition to being the smallest subunit of CAF-1, dCAF-1-p55 is also detected in several other complexes, including Nucleosome Remodeling Factor (NURF), Polycomb Repressive Complex 2 (PRC2), Sin3-HDAC1 complex and dREAM (*Drosophila* RBF, E2F, and Myb-interacting proteins) [8, 22-24]. As a member of the WD40 family protein, dCAF-1-p55 can bind directly to histones H3 and H4 [25-26]. Some of the complexes that contain dCAF-1-p55 have been reported to function in genomic stability maintenance. Mutation of the largest subunit of CAF-1 compromises the pericentric heterochromatin maintenance [6, 27]. One of the components of Sin3-HDAC1 complex, Sds3, has been shown to be required for proper pericentric heterochromatin formation and chromosome segregation [28]. Loss of RBF (as well as its mammalian homolog pRB) leads to centromere dysfunction and chromosomal instability [29-31]. Moreover, dCAF-1-p55 has been found to participate in the process of CID assembly *in vitro*, which is crucial for proper chromatids separation [13-15]. All these studies point to a possibility that dCAF-1-p55 plays a role in the maintenance of chromosome stability.

Recently, two groups described their *dCAF-1-p55* mutants that were obtained by EMS mutagenesis and imprecise P-element excision respectively, showing the function of dCAF-1-p55 in cell survival and patterning during development [32-33]. Our results show that *dCAF-1-p55* homozygous mutants are lethal at the larval stage with an apparent developmental delay. About 45% of the mutants can survive for more than ten days as larvae before death, a period more than two times longer than the wild type larvae. Compared with the control larvae, the body sizes of *dCAF-1-p55* mutants are much smaller. Chromosome spreads of mitotic neuroblasts showed that in *dCAF-1-p55* mutant cells the sister chromatids association becomes partially defective. Moreover, the frequency of lagging chromosomes at the metaphase plane increases in anaphase of *dCAF-1-p55* deficient cells. Our study suggests that dCAF-1-p55 plays an important role in the maintenance of chromosomal stability during development.

1 Materials and Methods

1.1 Fly stocks and genetics

Flies were raised at 25℃ for all experiments. The stocks used in this study were listed below:

y w
y w; Sp/CyO; MKRS/TM2,y^+
y w; ey-FLP; MKRS/TM2, y^+
y w; hs-I-Sce I, hs-FLP, Sco/CyO
w^{1118}; hs-I-Cre I, Sb/TM6B
w; actin-Gal4
y w; $p55^{7\text{-}15a}$ /TM3, Kr-GFP
y w; $p55^{7\text{-}15a}$ /TM6B, Tb
y w; UAS-p55/CyO

1.2 DNA constructs

5.6kb *dCAF-1-p55* genomic fragment (2.8kb upstream and downstream from the ATG start codon respectively) with designed modifications was cloned into the *pTARG* vector essentially as previous described [34-39] to get the gene targeting donor construct *pTARG-p55**. The mutations were introduced by PCR with oligos (underlined are restriction sites and *italic* are the *Not* I cutting sequences which were used to replace the original ATGGTGG) listed below. The primers for the upstream 2.8kb fragment were 5'-AAAAACTGCAGTAAACAGCCGGAACC-3' and 5'-GCTGCGAT*GCGGCCGC*TTCGAGGCTTTTAGGCAC-3' while the primers for the downstream 2.8kb were 5'-GCCTCGAA*GCGGCCGC*ATCGCAGCGATAATGGTA-3' and 5'-ATTGCACTAGTAGGCCAACGAGGACA-3'. A *Not* I restriction site which was used for mutation

identification was generated by substituting ATGGTGG with GCGGCCGC (Fig. 1a). Oligos used to introduce the I-*Sce* I recognition sequence at the *Mfe* I cutting site were 5'-AATTTAGGGATAACAGGGTAAT-3' and 5'-AATTATTACCCTGTTATCCCTA-3'.

To construct *pUAST-p55*, cDNA fragment of *dCAF-1-p55*, which was retrieved by digestion of *pGEX-p55* (kindly provided by Dr. J. Tyler, see reference [8] for details) with *EcoR* I and *Xho* I, was put into the *pUAST* vector at the same restriction sites.

1.3 Generation of the *dCAF-1-p55* mutant

The *dCAF-1-p55* mutants were generated by ends-in gene targeting technique [34-40]. Donor transgenic flies bearing *pTARG-p55** on the second chromosome were crossed to *y w; hs-I-Sce I, hs-FLP, Sco/CyO* flies. Heat shocks (38℃, 1 hr each) were applied to the progenies for three times on the days 2nd, 3rd and 4th day respectively after egg laying. Virgins were singly crossed to *y w; ey-FLP; MKRS/TM2,* y^+ males, and the offspring were screened for targeted integration of targeting construct indicated by the w^+ marker. The targeted alleles were then crossed to w^{1118}*; hs-I-Cre I, Sb/TM6B* for the reduction of two *dCAF-1-p55* copies (one wild type and one mutant copy). The descendants were heated shocked at 36℃ for 1 hr at the third instar larval stage. w^- males were crossed singly to *y w; MKRS/TM6B, Sb* to make stocks. To identify the potential mutant alleles, a pair of primers (5'-TCGACAATCTTTAGCATTGC-3' and 5'-AACGCTGGCGATGAGTAGAT-3') was used to amplify a 600bp fragment. *Not* I digestion of the 600bp PCR products yields two 300bp fragments when the designed mutations are introduced into the genome. One of the alleles, designated $p55^{7\text{-}15a}$, further confirmed by DNA sequencing for the intended mutations was molecularly characterized and used throughout this study.

To verify the expression of dCAF-1-p55 protein in $p55^{7\text{-}15a}$ mutants, western blot analysis was carried out according to the standard procedure [35-37, 41]. Briefly, the third instar larvae with correct genotypes were collected, and proteins were extracted with radioimmunoprecipitation assay buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, and 1 mM phenylmethylsulfonyl fluoride) and separated by SDS-10% polyacrylamide gel electrophoresis (PAGE). The antibodies used are: rabbit anti-p55 (1:5000; a kind gift from Dr. J. Tyler [8]) and mouse anti-tubulin (1:5000, purchased from Kwbiotech).

1.4 Statistical analysis

Homozygous mutants (*y w;* $p55^{7\text{-}15a}$) were selected from *y w;* $p55^{7\text{-}15a}$*/ TM3, Kr-GFP* stock at the early embryonic stage using the GFP-marked balancer chromosome. The homologous and heterozygous animals were transferred independently into fresh medium and counted carefully at the indicated time windows (Fig. 2a and 2b).

1.5 Chromosome spreads

For metaphase chromosome spreads, brains from *y w* and *y w;* $p55^{7\text{-}15a}$ homozygous third instar larvae were dissected in PBS, treated with colchicines and hypotonic solution, fixed in acetic acid/methanol/H_2O (11v:11v:2v) and stained for 5 min in 0.2 mg/ml DAPI. Anaphase chromosome spreads were carried out in the same way without colchicine treatment. The preparations were examined and photographed under a Nikon 90*i* fluorescent microscope (Nikon; Tokyo, Japan).

1.6 Immunohistochemistry

The preparations of anaphase chromosome spreads were washed for three times in PBST before incubated with Rabbit anti-phosphohistone H3 (Ser 10) antibody (1:100, Millipore) overnight. After three washes with PBST, FITC-conjugated anti-rabbit secondary antibody (1:100, Jackson ImmunoResearch Laboratories) was used with a dilution of 1:100 for 2 hrs at the room temperature, followed by DAPI staining. Images were taken using a Nikon 90*i* fluorescent microscope (Nikon; Tokyo, Japan).

2 Results

2.1 Generation of $p55^{7\text{-}15a}$ mutants

To investigate the function of *dCAF-1-p55 in vivo*, we set off to generate *dCAF-1-p55* mutant flies with the

ends-in gene targeting technique (Fig. 1a). At first, a modified ~5.6 kb *dCAF-1-p55* genomic fragment was obtained by PCR. This fragment contained a replacement of the original **ATG**GTGG (**ATG** is the start codon of *dCAF-1-p55* gene) with the *Not* I recognition sequence GCGGCCGC, and an inertion of I-*Sce* I recognition sequence about 1 kb downstream of the ATG. The DNA fragment with intended modifications was cloned into the *pTARG* vector to get the gene targeting construct *pTARG-p55**. Through microinjection and following single crosses, we obtained the donor transgenic flies containing *pTARG-p55**. Taking advantage of this donor fly stock and the endonuclease I-*Sce* I, a genetic screen helped us finally get several lines of the mutants with the translation start codon mutation and a frame shift in the coding sequence of *dCAF-1-p55* gene (Fig. 1a and 1b). One of the alleles, $p55^{7\text{-}15a}$, were identified first by *Not* I digestion (Fig. 1c). The 600bp PCR products from the wild type genomic DNA cannot be cut by *Not* I while the PCR products of the $p55^{7\text{-}15a}$ mutants were cut into two 300bp fragments, which indicated that the designed mutations were successfully introduced into the *dCAF-1-p55* gene. Subsequent sequencing results further confirmed the ATG mutation and frame shift (Fig. 1b). Western blot result showed the loss of dCAF-1-p55 protein expression in $p55^{7\text{-}15a}$ homozygous mutants (Fig. 1d). Together, we conclude that $p55^{7\text{-}15a}$ is a null allele of the *dCAF-1-p55* gene and thus all the experiments described below were carried out with this allele.

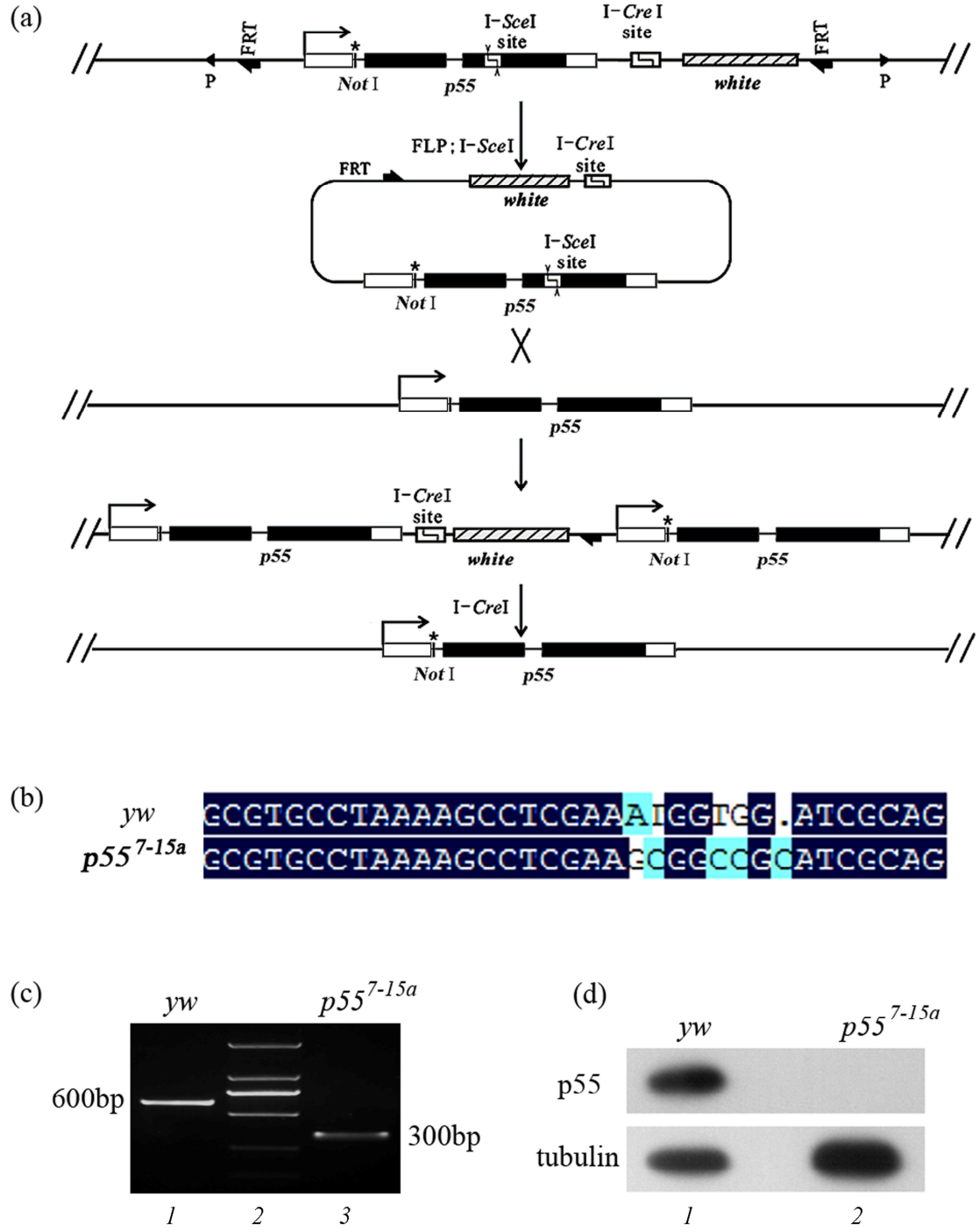

Fig. 1 Generation and molecular characterization of the *p55*$^{7\text{-}15a}$ allele

(a) Schematic diagram of the *dCAF-1-p55* genomic locus and the gene targeting strategy. The modified *dCAF-1-p55* and the marker gene, *mini-white,* are circularized from the transgenic genome by FLP recombinase and linearized by the yeast restriction endonuclease I-*Sce* I. Then *dCAF-1-p55* locus is duplicated (one wild type and the other mutant) as a result of 'ends-in' recombination of the targeting DNA and the resident *dCAF-1-p55* locus. For the reduction process, the duplicated DNA is cut by another endonuclease I-*Cre* I and then repaired by endogenous homologous recombination, which leads to a single copy of *dCAF-1-p55*. The mutant can be selected by the loss of *white* marker gene. * indicates the designed mutation site at the translation start codon. (b) Sequence alignment of the control (*y w*) and the mutant *p55*$^{7\text{-}15a}$ (*y w; p55*$^{7\text{-}15a}$) flies. Note that ATGGTGG has been substituted by *Not* I cutting site GCGGCCGC, yielding a frame shift in the coding sequence of *dCAF-1-p55*. (c) *p55*$^{7\text{-}15a}$ mutant identification by PCR in combination with *Not* I digestion (see materials and methods for details). The 600bp PCR products from the control and the *p55*$^{7\text{-}15a}$ mutants were digested by *Not* I. While the control 600bp DNA is resistant to *Not* I (lane 1) digestion, the PCR product of *p55*$^{7\text{-}15a}$ is cut into 300bp fragments (Lane 3). Lane 2 is DNA ladders. (d) Western blot analysis to detect the protein of dCAF-1-p55 expressed in wild type and the mutant of *p55*$^{7\text{-}15a}$. The 55 kDa product of *dCAF-1-p55* that is detected in the wild type, is absent in *p55*$^{7\text{-}15a}$ mutants (lane 2). Tubulin abundance was shown as a loading control.

2.2 *dCAF-1-p55* loss-of-function leads to developmental delay and lethality

In consistence with two very recent reports [32-33], the *dCAF-1-p55* homozygous mutants, $p55^{7\text{-}15a}$, are larval lethal and die before pupation. In order to more precisely characterize the developmental defects caused by *dCAF-1-p55* loss-of-function, we separated homozygotes from heterozygous $p55^{7\text{-}15a}$ animals at the early embryonic stage and monitored their developmental processes independently. The survival ratio of homozygous mutants decreased with time during the entire larval stage, with a sharp drop at the ninth day AEL (after egg laying). About 75% of *dCAF-1-p55* homozygous mutants survived until the 8th day AEL and about 30% could survive until the 12th day AEL without pupa formation. All of the mutants eventually died before the 15th day AEL (Fig. 2a and 2b). Apparently, the homozygous mutants have a much longer developing time at the larval stage than the wild type time (heterozygous animals) (Fig. 2a and 2b). Furthermore, at the same time window of development, for example 120h AEL, the homozygous mutants are significantly smaller in size than their heterozygous siblings (Fig. 2c).

To test whether the lethality of the homozygous mutants is specifically attributed to the loss of dCAF-1-p55, we performed a rescue experiment using the GAL4-UAS system. To achieve this, a *UAS-p55* transgenic fly stock was generated with the construct *pUAST-p55*, which contained the full length of *dCAF-1-p55* cDNA. Under the control of *actin-Gal4*, which is expressed ubiquitously in all the cells of *Drosophila*, expression of *UAS-p55* could successfully rescue the mutants to develop into adult flies. These results demonstrated that *dCAF-1-p55* is essential for normal *Drosophila* development.

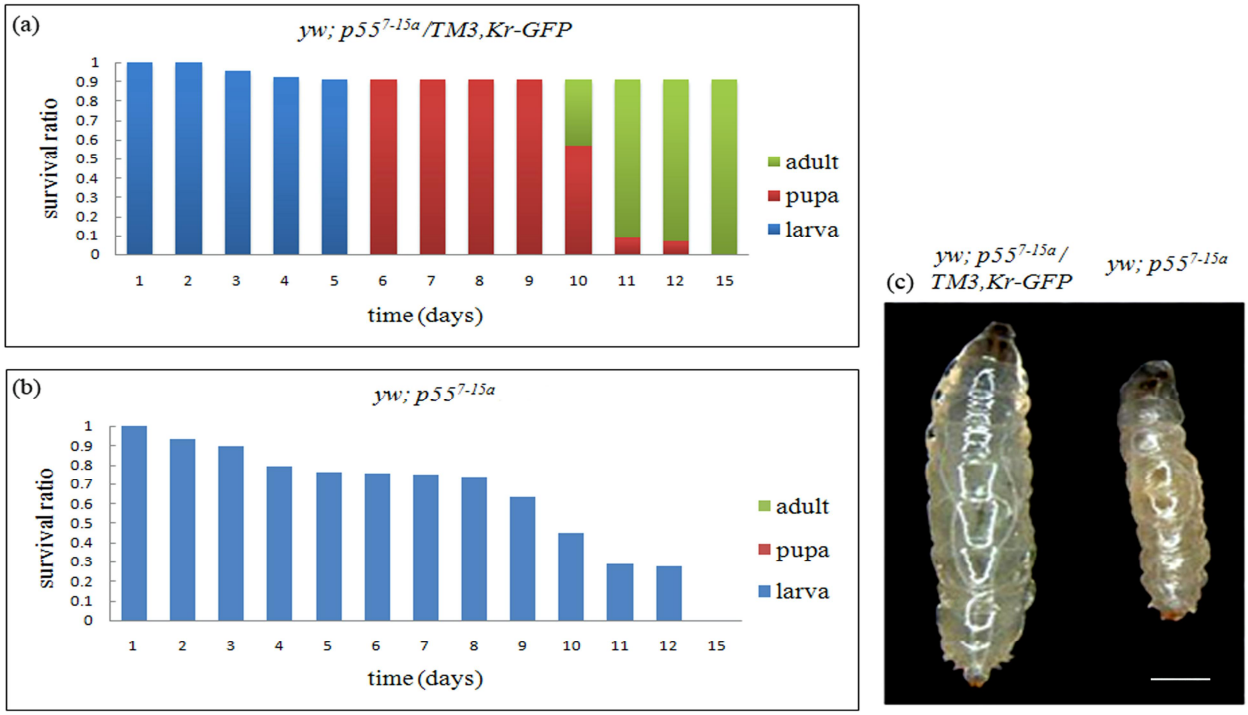

Fig. 2 *dCAF-1-p55* mutation led to developmental delay at the larval stage and lethality of the pupae in *Drosophila*

(a), (b) Survival ratio of $p55^{7\text{-}15a}$ hetrozygotes (a, as control) and homozygotes (b) throughout the development from the larval stage to the adults. The control flies exhibited a normal developmental time course (a) while all of the $p55^{7\text{-}15a}$ mutants died before pupation with a much longer larval stage (b). (c) $p55^{7\text{-}15a}$ mutants showed a significant development delay that rendered a smaller body size compared with their heterozygous siblings at 120h AEL. Scale bar: 0.5 mm.

2.3 *dCAF-1-p55* is required for proper sister chromatids association

dCAF-1-p55 is known to be involved in multiple protein complexes including those that function in DNA replication, histone deposition and chromatin remodeling [8, 22-24]. To clarify whether dCAF-1-p55 plays a role in the maintenance of genome stability, we examined the metaphase spreads of neuroblasts from third instar larvae of *dCAF-1-p55* homozygous mutants. The brains of the third instar larvae were treated with colchicine to arrest cells in the metaphase before stained with DAPI to identify chromosomes. As shown in Fig. 3, *dCAF-1-p55* mutant cells exhibited abnormally condensed metaphase chromosomes compared with the wild-type cells. In wild type cells, the

highly condensed chromosomes appeared to be short and compact in the metaphase (Fig. 3a). However, in *dCAF-1-p55* mutant cells, the chromosome arms were longer and more loosened than in the controls. Moreover, abnormal sister chromatids association was also observed in the *dCAF-1-p55* mutant cells, likely due to the abnormally assembled centromeres in the absence of dCAF-1-p55. Broken chromosomes could be found more frequently in *dCAF-1-p55* mutant cells than in wild type cells (Fig. 3b). These results suggested that *dCAF-1-p55* is important for metaphase chromosome condensation and sister chromatids association, which is important for maintaining chromosomal stability.

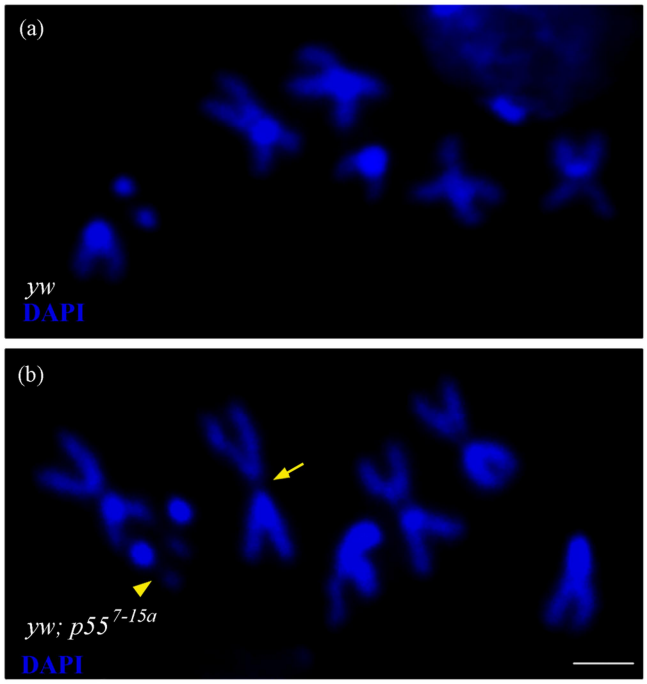

Fig. 3 *dCAF-1-p55* loss-of-function caused defective sister chromatids association

(a), (b) DAPI-stained metaphase spreads of neuroblasts from the third instar larvae. (a) Normal pattern of metaphase chromosomes from wild type cells. (b) Typical chromosome spreads of $p55^{7\text{-}15a}$ mutants. A large fraction of the chromosome arms became longer than the wild type and sister chromatids were precociously separated with decondensed centromeres (arrow). The broken chromosomes could also be frequently observed in the mutant cells (arrow head). Scale bar: 2 μm.

2.4 *dCAF-1-p55* is required for chromosome segregation during the anaphase

Another criteria for chromosome instability is that the anaphase cells do not distribute evenly the duplicated sets of chromosome in to daughter cells during mitosis, in other words, these cells are defective in chromosome segregation. To further confirm the potential role of *dCAF-1-p55* in maintaining chromosome stability, we examined the anaphase chromosome spreads of *dCAF-1-p55* mutants. In higher eukaryotic cells, the sister chromatids separate from each other and move toward the spindle poles during anaphase. Lagging chromosomes are defined as chromosomes or chromatids which still stay at metaphase plate when the cells enter anaphase [42]. In our experiments, the mitotic cells were indicated by phosphorylated histone H3 (pH3) (Fig. 4b and 4d). Compared with the wild type animals, the *dCAF-1-p55* mutants had significantly more mitotic cells that contain lagging chromosomes. Fig. 4e shows the ratio of the cells that contain lagging chromosomes in the total number of anaphase cells of each brain. More than 70% of the anaphase cells of the *dCAF-1-p55* mutant contain lagging chromosomes, while it is only 17% in wild type brains. This observation of increase of lagging chromosomes in the *dCAF-1-p55* mutant cells is likely associated with results of sister chromatids association defects (see 2.3 and Fig. 3 for details) that were described in the last section. Together, these results demonstrated that *dCAF-1-p55* is essential for the maintenance of chromosome stability in *Drosophila*.

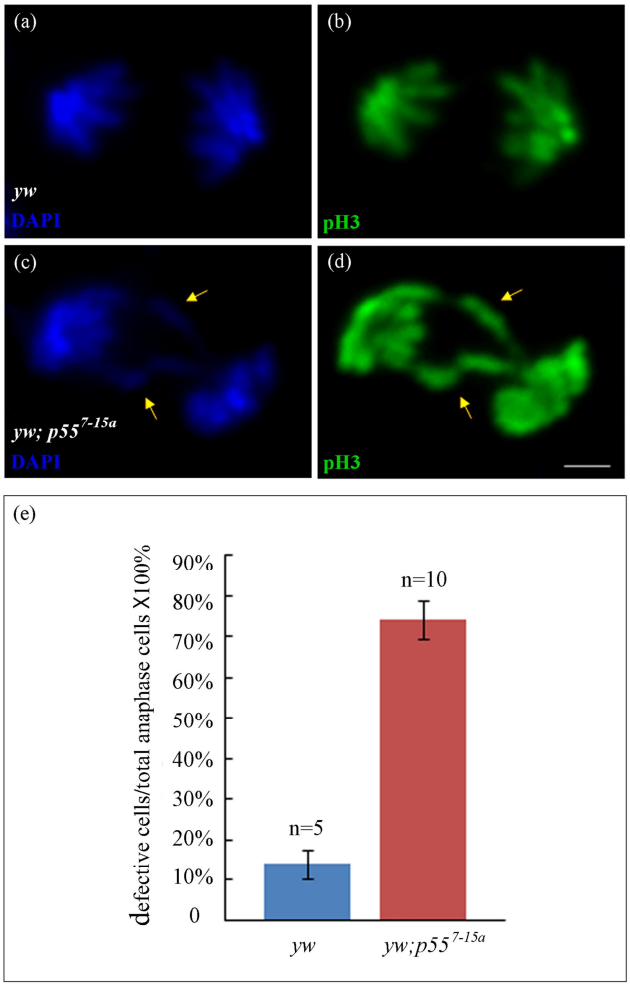

Fig. 4 *dCAF-1-p55* mutation resulted in abnormal segregation behavior of the anaphase chromosomes

(a), (b) Anaphase chromosome spreads of wild type cells showing normal segregation pattern. (c), (d) $p55^{7\text{-}15a}$ cells exhibit defective segregation behavior of the chromosomes in anaphase. Yellow arrows indicate lagging chromosomes around the metaphase plate. The fractions of anaphase cells that contain lagging chromosomes (defective cells) in wild type cells and *dCAF-1-p55* mutants are shown in (e). The numbers of brains scored for statistic analysis are indicated on the top of each column. Scale bar: 2 μm.

3 Discussion

dCAF-1-p55 has been shown to be a multifunctional gene whose product appears in a handful of protein complexes [8, 22-24]. However, the precise functional mechanisms of this gene remain elusive. Recently, two groups reported *Drosophila dCAF-1-p55* mutants which were obtained via EMS screen and imprecise P-element excision respectively. The mutant generated by Wen and colleagues did not show any reduction of histone H3K27 tri-methylation in which is in contadiction with the report by Anderson and colleagues with another mutant [32-33]. However, both of the mutants described in their papers are leathal suggesting the essentiality of *dCAF-1-p55* for *Drosophila* development [32-33]. In this study, we report a new allele of *dCAF-1-p55* in *Drosophila* that were generated by gene targeting [34-40]. Mutation of *dCAF-1-p55* led to animal lethality and significant developmental delay. More importantly, defects of sister chromatids association and anaphase chromosome segregation in the *dCAF-1-p55* mutant cells were described here for the first time.

All the results described in our study suggests that *dCAF-1-p55* may play a role in chromosomal stability maintenance. Our findings are in agreement with the following lines of facts about *dCAF-1-p55*. (1) As a member of the WD40 family protein, dCAF-1-p55 is involved in multiple complexes that function in histone deposition,

chromatin remodeling and centromere assembly [8, 22-24]. All these complexes are important for the normal chromatin structure and genomic stability maintenance. For instance, the disruption of *dCAF-1-p180*, the largest subunit of dCAF-1 compromises the pericentric heterochromatin formation and/or maintenance [6, 27]. Sin3-HDAC1 is another dCAF-1-p55-containing chromatin remodeling complex, one component of which, Sds3 has been reported to control the pericentric heterochromatin-specific modifications to ensure proper pericentric heterochromatin formation and chromosome segregation [28]. (2) Mutations of the cell cycle regulating proteins, Myb and RBF, both of which interacts with dCAF-1-p55, caused defects in chromosome condensation and association [29-31]. (3) dCAF-1-p55 was found as a component of CID assembly complex (CID/H4-p55) in an *in vitro* experiment [13-15]. It was reported that both purified and reconstituted CID/H4-p55 are sufficient for centromeric chromatin assembly [13-15]. Therefore, we speculate that the chromatids association and segregation defects caused by *dCAF-1-p55* loss-of-function may be due to an altered chromatin structure, deregulated cell cycle control and/or the failure of centromere assembly.

Chromosomal stability maintenance is important for the development of all organisms. When the stability is challenged, cells may lose or gain some of the chromosomes (or fragments of the chromosomes) of their parental cells during cell division and subsequently become aneuploidy [43]. Recent studies have shown that aneuploidy and chromosomal instability (CIN) play causal role in tumorigenesis [31, 44-46]. Our studies suggest that *dCAF-1-p55* loss-of-function may lead to CIN, but the possibility to cause aneuploidy still needs further investigations. Nevertheless, our results provide a potential link between dCAF-1-p55 and cancer development. This prediction is in line with the observation that altered RbAp46/48 expression levels are found in various tumor cells [18-21].

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黑腹果蝇 *dCAF-1-p55* 突变引起果蝇发育迟缓和染色体不稳定性*

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摘要 在真核生物中高度保守的染色质装配因子 1（chromatin assembly factor 1，CAF-1）是染色质装配过程中的组蛋白分子伴侣之一．dCAF-1-p55 是果蝇中 CAF-1 复合物中的最小亚基，它与另外两个亚基 dCAF-1-p180 及 dCAF-1-p105 一起负责将组蛋白 H3/H4 组装到新合成的 DNA 上．除了 CAF-1 复合物，dCAF-1-p55 还参与其它多个复合物的形成，如 NURF，PRC2 及 Sin3-HDAC1．dCAF-1-p55 的这一广泛参与性提示了其功能的多样性和重要性．为了研究 dCAF-1-p55 的体内功能，我们利用基因靶向敲除技术制备了果蝇 *dCAF-1-p55* 突变体.实验结果表明，*dCAF-1-p55* 的缺失导致果蝇发育迟缓并且最终致死.进一步研究发现，在 *dCAF-1-p55* 突变细胞中，中期染色体较为松散，姐妹染色单体连接异常，后期染色体不能正常分离．这些缺陷都是与癌症发生密切相关的染色体不稳定性（chromosome instability，CIN）的典型特征．综上所述，我们的研究表明了 *dCAF-1-p55* 在发育过程及维持染色体稳定性方面的重要作用，同时提示该基因具有保护细胞免遭 CIN 和癌变的潜在功能．

关键词 果蝇，dCAF-1-p55，染色体不稳定性，染色体分离，姐妹染色单体连接

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